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Effect of Chronic Treatment with Estrogen on the Dipsogenic Response of Rats to Angiotensin[1]

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FREGLY, M. J. *Effect of chronic treatment with estrogen on the dipsogenic response of rats to angiotensin.* PHARMAC. BIOCHEM. BEHAV. 12(1) 131–136, 1980.—Female rats treated with estradiol benzoate for 23 weeks at doses of 25 and 44 μg/kg/day had an attenuated drinking response to peripheral administration of graded doses of angiotensin I and angiotensin II. No significant difference between the responses of the two estrogen-treated groups was observed, suggesting that maximal attenuation had been attained with the lower dose. Angiotensin II (50, 100 and 200 μg/kg, IP) increased urine output and urinary sodium excretion rate in a graded fashion with increasing doses in both control and estrogen-treated rats. However, the latter had a somewhat greater response. Administration of angiotension I (50, 100 and 200 μg/kg, SC) had a similar, but less consistent, effect on urine output and urinary sodium excretion rate in both control and estrogen-treated groups. The attenuated drinking response of estrogen-treated rats to angiotensin I and angiotensin II suggests either that administered angiotensin failed to gain access to the brain or that central receptors mediating thirst are less sensitive in estrogen-treated rats. The present studies fail to distinguish between these possibilities.

Thirst Angiotensin I Angiotensin II Estradiol benzoate Water intake Urine output Urinary sodium

RATS treated chronically with either estradiol benzoate or ethynyl estradiol have an attenuated drinking response when either isoproterenol or angiotensin II is administered [6,16]. A dose-response relationship between water intake during the first hour after treatment and dose of isoproterenol administered revealed a significantly depressed slope in the case of the estrogen-treated group [16]. A similar dose-response relationship for angiotensin II was not determined in the earlier study [6]. It was the objective of the present studies to establish a dose-response relationship between dose of either angiotensin I or angiotensin II administered and water intake during the first hour after administration of the compounds in rats treated chronically with estradiol benzoate.

METHOD

Experiment 1: Effect of Angiotensin II on Water Intake of Estrogen-Treated Rats

Eighteen female rats of the Blue Spruce Farms (Sprague-Dawley) strain were used. They were kept 3 per cage in a room maintained at 25 ± 1°C and illuminated from 0800 to 1800 hr. All rats received tap water and Purina Laboratory Chow ad lib.

Six of the rats were untreated, six received 25 μg estradiol benzoate/kg/day and an additional six received 44 μg estradiol benzoate/kg/day. All treated rats received estrogen for 23 weeks prior to this experiment.

Estradiol benzoate was administered via Silastic tubes. Silastic tubing (no. 602-231, 0.25 mm wall thickness, 10 mm long) containing crystalline estradiol benzoate was implanted SC between the shoulder blades of 6 rats while twice the length of the same tubing containing estradiol benzoate was implanted SC in a second group of 6 rats. The third group of 6 rats was implanted SC with a 10 mm length of empty Silastic tubing. Dimethylpolysiloxane (Silastic) tubing has been shown to allow diffusion of certain crystalline steroids into various media at a constant rate over relatively long periods of time [4,9]. Previous experience in this laboratory has indicated that this method of steroid administration provides a reliable means of achieving reasonably constant drug release for periods up to 6 months [16].

At 0900 hr on the day of the first study, all rats were administered 1 ml isotonic saline/kg body weight IP. Each rat was then placed in an individual stainless steel metabolism cage and given a preweighed water bottle consisting of an infant nursing bottle with a cast aluminum spout as described by Lazarow [10]. Water intakes were measured hourly for 3 hours thereafter. In addition, urine output was measured hourly for 3 hours. The 3 hour urine sample from each rat was pooled and the urinary output of sodium and potassium measured by flame photometry.

Four days after completion of this study, the first study

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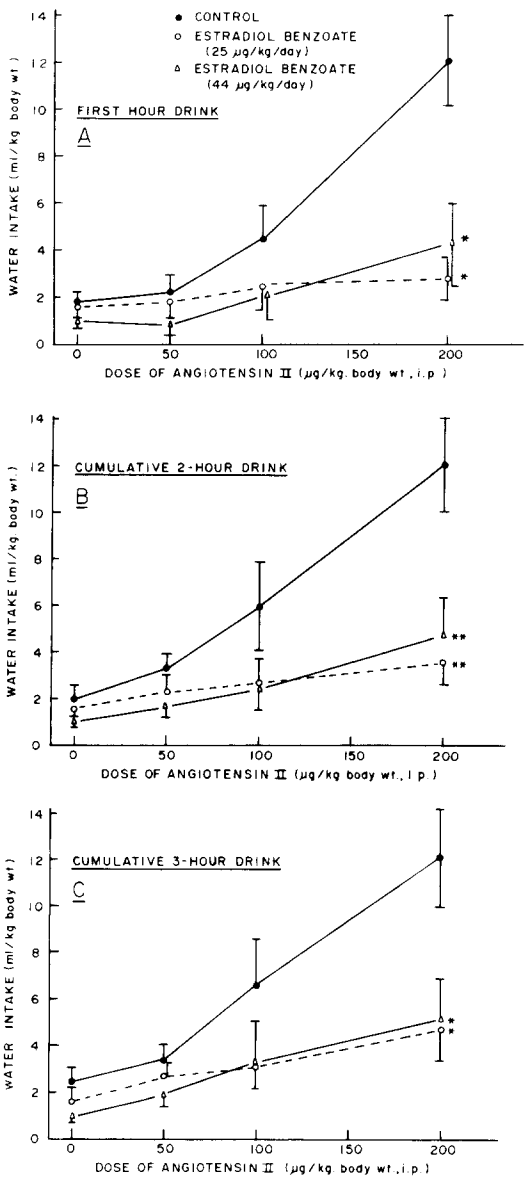

FIG. 1. Effect of acute IP administration of graded doses of angiotensin II on cumulative water intake during the first (A), second (B) and third (C) hours of the study. Symbols for control and the two estrogen-treated groups are designated in the figure. One standard error is set off at each mean.

using angiotensin II (U.S. Biochemicals Corp., Cleveland, Ohio 44122) (50 μg/kg body weight IP) as the dipsogen was begun. The procedure was identical to that described above. At 4 day intervals thereafter angiotensin II at doses of 100 and 200 μg/kg body weight, was administered IP. These studies were also carried out in an identical fashion to that described above.

A one way analysis of variance was used to analyze the data resulting from each study [15]. Comparison between individual groups was made by Student's t test using the pooled variance from the analysis of variance [7]. Significance was set at the 95% confidence limit. A linear regression analysis of water intake versus logarithm of dose of angiotensin II administered was also carried out using a Hewlett-Packard computer model 10 and program II-5.

Experiment 2: Effect of Angiotensin I on Water Intake of Estrogen-Treated Rats

The same rats used in Experiment 1 were used in Experiment 2. This experiment began two weeks after completion of Experiment 1. The only difference from Experiment 1 was that angiotensin I (U.S. Biochemicals Corp., Cleveland, Ohio 44122), rather than angiotensin II, was used. The doses administered were 50, 100 and 200 μg/kg SC.

At the end of this experiment, each rat was anesthetized with ether and the tube containing estradiol benzoate was removed from its subcutaneous site. After cleaning each tube of adherent tissue, it was placed in a vacuum desiccator for 72 hours and then weighed on an analytical balance. Tubes from the group in which the 10 mm tube was implanted had a mean weight loss of 7.1 ± 0.2 μg/day while the mean weight loss from the 20 mm tube was 11.6 ± 0.8 μg/day. When calculated on the basis of mean body weight during the time the tubes were implanted, weight losses of 25 and 44 μg/kg/day occurred from the tubes.

Statistical analysis of the data was the same as that described in Experiment 1. Regression analysis of water intake versus logarithm of dose of angiotensin I administered was carried out as described in Experiment 1.

RESULTS

Experiment 1

Administration of graded doses of angiotensin II increased water intake in a graded fashion in the control group during all three hours of the study (Fig. 1). In contrast, rats treated with either 25 or 44 μg estradiol/kg/day had a significantly depressed response.

Linear regression analysis of water intake versus the logarithm of the dose of angiotensin II administered revealed for the control group correlation coefficients of 0.71, 0.68 and 0.66 for the first three hours respectively of the study ($p<0.01$). The groups given estradiol benzoate had correlation coefficients varying from 0.43 to 0.47 ($p<0.05$) for the first three hours of the study. Comparison of the slopes and intercepts of the linear regressions of the three groups during the second and third hours of the study revealed a significant ($p<0.01$) reduction from the control group in the slopes of the two estrogen-treated groups compared to the control group. However, treatment with estradiol benzoate had no significant effect on the intercepts of the lines.

Administration of graded doses of angiotensin II increased urine outputs of both control and estrogen-treated groups during all 3 hours (Table 1). The urine outputs during the second and third hours were somewhat greater for estrogen-treated rats than for controls. Treatment with angiotensin II at 100 μg/kg increased urine output of both estrogen-treated groups significantly above the level of the control group during the second and third hours of the study (Table 1). When 50 μg angiotensin II/kg was administered, urine outputs of the estrogen-treated groups were increased above that of controls during the last two hours of this study but output was increased significantly ($p<0.05$) only for the group administered the highest dose of estrogen and only during the third hour of the study. In contrast, administration of the highest dose of angiotensin II (200 μg/kg) failed to

TABLE 1

EFFECT OF ACUTE ADMINISTRATION OF ANGIOTENSIN II ON WATER INTAKE, URINE OUTPUT AND URINARY ELECTROLYTE EXCRETION IN RATS TREATED CHRONICALLY WITH ESTRADIOL BENZOATE

Experimental group	No. of rats	Mean body wt (g)	Cumulative water intake (ml/kg body wt) during: 1	2	3 hr	Cumulative urine output (ml/kg body wt) during: 1	2	3 hr	Rate of urinary excretion (mEq/kg/3hr) of: Na	K	Urinary Na/K ratio
Saline administration (IP)											
Control	6	264 ± 6	1.8 ±0.4	2.0 ±0.5	2.5 ±0.6	2.5 ±0.8	3.6 ±0.8	4.1 ±0.9	0.29 ±0.15	0.42 ±0.18	0.69 ±0.13
Estradiol benzoate (25 μg/kg/day)	6	256 ± 5	1.6 ±0.6	1.6 ±0.6	1.6 ±0.6	0.1 ±0.1	7.3 ±2.2	8.6 ±1.5	0.79 ±0.13	0.95 ±0.10	0.83 ±0.09
Estradiol benzoate (44 μg/kg/day)	6	250 ± 4	0.9 ±0.1	1.0 ±0.1	1.0 ±0.1	0.0 ±0.0	5.9 ±1.3	8.4 ±1.0	0.79 ±0.09	0.97 ±0.09	0.81 ±0.08
Angiotensin II (50 μg/kg body wt, IP)											
Control	6	286 ± 6	2.2 ±0.7	3.2 ±0.6	3.4 ±0.6	2.7 ±1.8	5.1 ±2.4	5.6 ±2.4	0.56 ±0.20	0.38 ±0.13	1.46 ±0.36
Estradiol Benzoate (25 μg/kg/day)	6	295 ± 4	1.8 ±0.7	2.3 ±0.7	2.7 ±0.7	4.7 ±1.7	10.9 ±2.1	13.1 ±1.7	1.04 ±0.21	0.74 ±0.13	1.41 ±0.46
Estradiol benzoate (44 μg/kg/day)	6	285 ± 9	0.9 ±0.5	1.7 ±0.5	1.9 ±0.5	1.9 ±1.3	7.4 ±1.1	13.8 ±1.8*	1.30 ±0.17*	1.10 ±0.20†	1.18 ±0.26
Angiotensin II (100 μg/kg body wt, IP)											
Control	6	302 ± 2	4.5 ±1.5	6.0 ±1.9	6.6 ±2.0	4.9 ±0.9	5.8 ±0.7	6.7 ±0.5	0.82 ±0.20	0.54 ±0.06	1.52 ±0.40
Estradiol benzoate (25 μg/kg/day)	6	309 ± 6	2.5 ±1.1	2.7 ±1.0	3.1 ±1.0	4.4 ±1.4	10.2 ±1.4*	11.0 ±1.3*	1.06 ±0.04	0.99 ±0.15	1.07 ±0.20
Estradiol benzoate (44 μg/kg/day)	6	283 ±11	2.1 ±1.1	2.6 ±1.1	3.3 ±1.7	4.7 ±1.6	11.7 ±0.9†	13.6 ±1.4†	1.39 ±0.31	0.64 ±0.17	2.17 ±0.52
Angiotensin II (200 μg/kg body wt, IP)											
Control	6	314 ± 6	12.1 ±2.0	12.1 ±2.0	12.2 ±2.0	4.1 ±1.0	9.0 ±2.4	12.1 ±1.8	1.02 ±0.12	0.59 ±0.10	1.73 ±0.53
Estradiol benzoate (25 μg/kg/day)	6	310 ± 6	2.8 ±0.9†	3.6 ±0.9†	4.7 ±1.3*	5.8 ±1.6	8.8 ±2.1	12.3 ±2.2	1.33 ±0.13	1.08 ±0.22	1.23 ±0.41
Estradiol benzoate (44 μg/kg/day)	6	284 ±10	4.2 ±1.7†	4.8 ±1.6†	5.1 ±1.7*	4.0 ±2.5	10.0 ±2.1	11.9 ±3.4	1.54 ±0.15	1.56 ±0.36	0.99 ±0.23

*Significantly different from control ($p<0.05$).
†Significantly different from control ($p<0.01$).

affect urine output of estrogen-treated rats above that of controls apparently because this high dose of angiotensin II nearly doubled the urine output of control rats above that accompanying administration of 100 μg angiotensin II/kg.

Administration of graded doses of angiotensin II to control rats was accompanied by a graded increase in urinary sodium, but not potassium, excretion (Table 1). Estrogen-treated rats generally excreted more sodium and potassium than controls at any dose of angiotensin administered but the excretion was significant for both only when 50 μg angiotensin II/kg was administered to the group receiving the highest estrogen dosage. The urinary sodium to potassium ratio of the control group appeared to increase with increasing doses of angiotensin II. This trend, however, was not apparent in the estrogen-treated group.

Experiment 2

Administration of graded doses of angiotensin I increased water intake in a graded fashion in the control group while increasing only slightly the water intake of the groups treated with either 25 or 44 μg estradiol/kg/day (Fig. 2A). Comparison of the slopes and intercepts of linear regressions of water intake versus the logarithm of the dose of angiotensin I administered to the three groups revealed a significant ($p<0.01$) reduction in the slopes of the two estrogen-treated groups compared to the control group. Treatment with estradiol benzoate had no significant effect on the intercepts of the lines.

In contrast to the effect of angiotensin II on urine output, angiotensin I at any dose administered had no greater effect on estrogen-treated rats than on controls. There was, how-

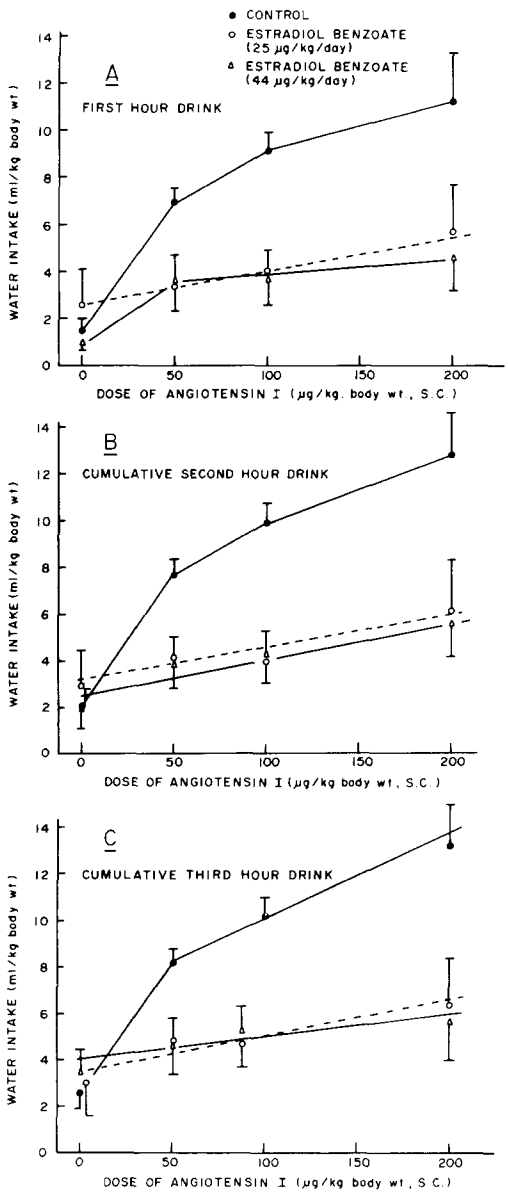

FIG. 2. Effect of acute SC administration of graded doses of angiotensin I on cumulative water intake during the first (A), second (B) and third (C) hours of the study. Symbols for control and the two estrogen-treated groups are designated in the figure. One standard error is set off at each mean.

ever, a tendency to increase urine output as the dose of angiotensin I increased (Table 2).

No effects of angiotensin I different from those seen in the control group were observed on urinary sodium and potassium excretion rate or urinary sodium/potassium ratio with either dose of estrogen although there was a tendency to increase urinary sodium and potassium outputs with increasing doses of angiotensin I in all groups (Table 2).

DISCUSSION

Rats treated chronically with estradiol benzoate had an attenuated drinking response both to angiotensin I and angiotensin II (Figs. 1 and 2). A regression analysis of the logarithm of the dose of either angiotensin I or angiotensin II administered on accumulative water intake during each of the 3 hours of the two studies showed a significant depression of the slope of the dose-response relationship for both angiotensin I and II in the two estrogen-treated groups. It can therefore be assumed that the attenuation of drinking observed in a previous study in which a single dose of angiotensin II (200 μg/kg) was administered to estradiol benzoate-treated rats is representative of other doses as well [16].

Some differences between the responses to angiotensin I and II were observed both in control and estrogen-treated rats. Thus, at a given dose of angiotensin I water intake of the control groups increased to a greater extent than with the same dose of angiotensin II (Tables 1 and 2). However, at a given dose of angiotensin II, urine output of the control group was greater than that observed for a comparable dose of angiotensin I. In the case of the estrogen-treated rats, angiotensin II appeared to have a greater effect on urine output than angiotensin I when compared at the same doses. It should be noted, however, that direct comparison of the responses to the two compounds is complicated by the fact that different routes of administration were used and that the experiments were designed only to compare the response of estrogen-treated rats with controls following administration of angiotensin I and II.

The effect of angiotensin II on urinary sodium excretion has been reported to be biphasic with low doses infused at 0.00005 to 0.005 μg/kg/minute always reducing urinary excretion of sodium while doses greater than 0.05 ug/kg/minute had a biphasic effect [1]. A transient reduction followed by a marked increase in urinary sodium occurred. In the present study angiotensin II, administered IP, increased urinary sodium output as the dose administered to control and estradiol-treated groups increased (Table 1). Thus, the doses used were apparently equivalent to, or exceeded, 0.05 μg/kg/minute by IV infusion. It is of interest that the estradiol-treated groups generally had a greater urinary sodium output than controls, even following administration of saline (Table 1). Estrogenic agents are reported to induce sodium retention in dogs [3,8] and to increase aldosterone excretion in rats [11]. In addition, chronic treatment with estrogens increases renin substrate concentration in blood of both rats and humans [2,13]. In view of these facts, it is difficult to explain the greater urinary sodium output by estrogen-treated rats. However, earlier studies from this laboratory support this observation [5]. Rats treated with ethynyl estradiol (41 μg/kg/day) for 5 weeks and given an IP load of isotonic NaCl solution (3% of body weight) excreted significantly more sodium during the 5 hr study than controls. The possibility that the renal tubule of the estrogen-treated rat is less sensitive to mineralocorticoid hormone is currently under study.

The doses of estradiol benzoate used in these studies did not result in a gradation of responses to administration of either angiotensin I or II. This suggests that the lower dose (25 μg/kg/day) produced a maximal attenuation and that nearly doubling the dose resulted in no greater effect. Thus, attenuation of the drinking response to angiotensin I and II apparently occurs at doses less than 25 μg/kg/day.

Earlier studies from this laboratory suggested that an attenuation of isoproterenol-induced drinking by estrogenic agents might occur primarily at the level of the receptor for

TABLE 2

EFFECT OF ACUTE ADMINISTRATION OF ANGIOTENSIN I ON WATER INTAKE, URINE OUTPUT AND URINARY ELECTROLYTE EXCRETION IN RATS TREATED CHRONICALLY WITH ESTRADIOL BENZOATE

Experimental group	No. of rats	Mean body wt (g)	Cumulative water intake (ml/kg body wt) during: 1	2	3 hr	Cumulative urine output (ml/kg body wt) during: 1	2	3 hr	Rate of Urinary excretion (mEq/kg/3 hr) of: Na	K	Urinary Na/K ratio
Saline administration (SC)											
Control	6	327 ± 9	1.5 ±0.5	2.2 ±0.7	2.6 ±0.7	1.6 ±0.6	3.1 ±0.8	4.5 ±1.5	0.47 ±0.18	0.60 ±0.18	0.78 ±0.09
Estradiol benzoate (25 μg/kg/day	6	304 ±10	2.6 ±1.5	2.9 ±1.5	3.1 ±1.5	2.6 ±1.5	7.0 ±3.0	7.9 ±2.7	0.61 ±0.16	0.82 ±0.17	0.77 ±0.13
Estradiol benzoate (44 μg/kg/day)	6	264 ±13†	0.9 ±0.1	2.1 ±0.9	3.4 ±1.1	0.7 ±0.5	7.4 ±1.7	9.4 ±0.9	0.73 ±0.07	0.83 ±0.13	0.88 ±0.14
Angiotensin I (50 μg/kg body wt, SC)											
Control	6	317 ± 5	7.0 ±0.6	7.7 ±0.6	8.2 ±0.6	1.2 ±0.7	1.6 ±0.7	4.1 ±1.7	0.22 ±0.08	0.44 ±0.13	0.50 ±0.11
Estradiol benzoate (25 μg/kg/day)	6	306 ± 4	3.4 ±1.1†	4.1 ±1.0†	4.8 ±1.0†	1.5 ±1.2	7.1 ±2.3	10.2 ±1.3	0.52 ±0.09	0.46 ±0.14	1.13 ±0.19
Estradiol benzoate (44 μg/kg/day)	6	278 ± 8†	3.5 ±1.5*	3.8 ±1.0†	4.7 ±1.0†	0.8 ±0.5	2.4 ±1.9	8.7 ±1.4	0.55 ±0.12	0.61 ±0.14	1.90 ±0.36
Angiotensin I (100 μg/kg body wt, SC)											
Control	6	307 ± 7	9.2 ±0.7	9.9 ±0.7	10.2 ±0.8	5.3 ±1.4	6.9 ±1.4	9.6 ±1.7	0.99 ±0.21	0.54 ±0.06	1.81 ±0.34
Estradiol benzoate (25 μg/kg day)	6	302 ± 5	3.9 ±1.0†	4.0 ±1.0†	4.7 ±1.0†	5.2 ±1.3	6.0 ±1.4	9.6 ±1.0	1.09 ±0.20	0.80 ±0.13	1.43 ±0.30
Estradiol benzoate (44 μg/day)	6	281 ± 7*	3.7 ±1.1†	4.2 ±1.1†	5.2 ±1.2†	3.7 ±1.0	4.6 ±1.2	7.3 ±1.4	0.91 ±0.18	0.97 ±0.14†	1.94 ±0.22
Angiotensin I (200 μg/kg body wt, SC)											
Control	6	330 ± 9	11.2 ±2.1	12.8 ±1.9	13.2 ±1.9	4.2 ±0.5	5.3 ±0.9	7.8 ±1.2	0.78 ±0.09	0.54 ±0.07	1.44 ±0.32
Estradiol benzoate (25 μg/kg/day)	6	306 ± 8	5.7 ±2.0	6.1 ±2.1*	6.4 ±2.0*	3.1 ±1.3	5.8 ±1.1	8.3 ±2.2	0.90 ±0.23	0.65 ±0.23	1.38 ±0.48
Estradiol benzoate (44 μg/kg/day)	6	267 ±18†	4.6 ±1.4	5.6 ±1.5*	5.6 ±1.5*	2.5 ±1.1	4.2 ±1.4	5.4 ±1.8	1.18 ±0.20	0.42 ±0.13	2.81 ±0.30

*Significantly different from control ($p<0.05$).
†Significantly different from control ($p<0.01$).

renin release [6,16]. While this aspect was not studied here, bypassing these receptors by administration of either angiotensin I or angiotensin II still resulted in an attenuated drinking response in estrogen-treated rats. A possibility exists that the administered angiotensin failed to enter the brain by way of the circumventricular organs in sufficient amount in estrogen-treated rats to stimulate thirst receptors [14]. An additional possibility is that the receptors for thirst in these areas are less responsive in estrogen-treated rats than are those of controls. Additionally, the possibility that a block may occur beyond the receptor must be considered. The present experiments do not distinguish among these possibilities which await additional experimentation.

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